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UNIVERSITY OF CALIFORNIA,  
IRVINE

The Spectral Phasor Approach as a Potential Diagnostic Tool for Breast Cancer

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCES

in Biomedical Engineering

by

Sam Pasin

Thesis Committee:

Professor Enrico Gratton, Chair  
Assistant Professor Michelle Digman  
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Thank you to the American Cancer Society, for their consent in using their information and data in the following introduction to this thesis.

## **ABSTRACT OF THE THESIS**

The Spectral Phasor Approach as a Potential Diagnostic Tool for Breast Cancer

By

Sam Pasin

Master of Sciences in Biomedical Engineering

University of California, Irvine, 2019

Professor Enrico Gratton, Chair

Breast cancer is a significant concern within the United States, causing over 200,000 new cases and 40,000 deaths each year (1). A new potential solution in the form of the spectral phasor approach to data analysis, paired with the noninvasive imaging approach of DOSI, presents itself as a safe, robust, and cost-effective, answering some of the concerns presented by current imaging techniques. By performing a Fourier transform to translate a stack of absorption coefficients at varying wavelengths to a single value or cluster of points on a spectral phasor plot, we are able to visualize the data in a clearly displayed format. In this paper, we explore the spectral phasor approach to analyze data gathered from both a single point on a patient, and multiple points in a grid on a patient as means to a diagnosis. Despite the potential that exists in the solution that this approach presents for quick and simple diagnosis, significantly more data is required to determine the reliability and reproducibility of this method.

## CHAPTER 1 - Introduction

Breast cancer refers to the formation of a malignant tumor in breast tissue. In the year 2017, there was an estimated 252,700 new cases of invasive breast cancer and 40,600 breast cancer related deaths in the United States [1]. There exists two forms of in situ breast cancer, or cancer that is confined to the site that it has originated. These types of in situ breast cancer are identified based on whether it forms in the ducts or the lobules of the breasts [2,3]. Breast cancer formed in the ducts, ductal carcinoma in situ (DCIS), occurs when abnormal cells replace the epithelial cells that line the ducts of the breasts [4]. The other main type of in situ breast cancer, lobular carcinoma in situ (LCIS), occurs when abnormal cells forms in the lobules, or milk-producing glands of the breasts [1,3]. Women with a history either DCIS or LCIS are about ten times more likely to develop an invasive breast cancer than women without [5,6]. Most cases of breast cancer, about 80%, are invasive or have spread beyond the area they have originated. There are up to 21 different types of invasive breast cancer, all of which vary in terms of cell morphology, growth, and architecture patterns [7].

As of January 2016, there were 3.5 million women in the United States with some history of breast cancer [8]. According to the

**Table 2. Age-specific Probability of Developing Invasive Breast Cancer for US Women**

Current age	10-year probability:	or 1 in:
20	0.1%	1,567
30	0.5%	220
40	1.5%	68
50	2.3%	43
60	3.4%	29
70	3.9%	25
<b>Lifetime risk</b>	<b>12.4%</b>	<b>8</b>

Note: Probability is among those free of cancer at beginning of age interval. Based on cases diagnosed 2012-2014. Percentages and "1 in" numbers may not be numerically equivalent due to rounding.

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Figure 1.1: Age-specific Probability of Developing Invasive Breast Cancer for US Women

This data table displays how lifetime risk of breast cancer increases as a woman ages, and therefore the concern of breast cancer is increased in older women.

American Cancer Society. Breast Cancer Facts and Figures 2017-2018. Atlanta: American Cancer Society, Inc.



**Table 1. Estimated New Female Breast Cancer Cases and Deaths by Age, US, 2017**

Age	In Situ Cases		Invasive Cases		Deaths	
	Number	%	Number	%	Number	%
<40	1,610	3%	11,160	4%	990	2%
40-49	12,440	20%	36,920	15%	3,480	9%
50-59	17,680	28%	58,620	23%	7,590	19%
60-69	17,550	28%	68,070	27%	9,420	23%
70-79	10,370	16%	47,860	19%	8,220	20%
80+	3,760	6%	30,080	12%	10,910	27%
<b>All ages</b>	<b>63,410</b>		<b>252,710</b>		<b>40,610</b>	

Estimates are rounded to the nearest 10. Percentages may not sum to 100 due to rounding.

©2017, American Cancer Society, Inc., Surveillance Research

Figure 1.2: Estimated New Female Breast Cancer Cases and Deaths by Age, US, 2017.

The number of new incidences and breast cancer deaths reported yearly, and their total percentage.

American Cancer Society. Breast Cancer Facts and Figures 2017-2018. Atlanta: American Cancer Society, Inc.

American Cancer Society, any given woman living in the United States has a 12.4%, or about one in eight, chance of being diagnosed with breast cancer at some point during her life [Figure 1.1].

Incidence rates of breast cancer increase with age in women until their 60s in which the rates begin to decrease, possibly to due to lower rates of screening and/or

incomplete detection in older women [Figure 1.2]. Both incidence and death rates of breast cancer in non-hispanic white and black women are slightly higher than those of women who are native american, hispanic, and asian/pacific islander [Figure 1.3]. In non-hispanic white women above the age of 60, the incidence rates of breast cancer are markedly higher than those of other demographic groups; however, non-hispanic black women have higher incidence rates before the age of 40 and are more likely to die from breast cancer at any given age [Figure 1.4]. These demographics come as a slight increase over the past four decades are assumed to be due to a number of various causes including changes in reproductive patterns, menopausal hormone use, rising prevalence of obesity, increased detection through screening, and even longer life expectancy [1].

During the 1980s and 1990s, the incidence rates of breast cancer in women rose

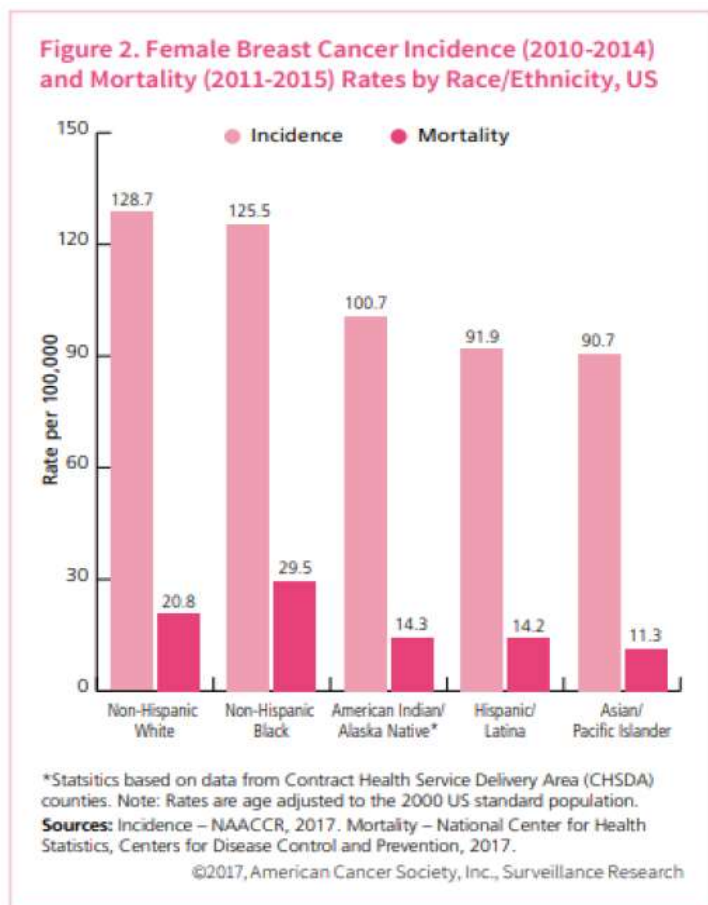


Figure 1.3: Female Breast Cancer Incidence (2010-2014) and Mortality (2011-2015) Rates by Race/Ethnicity, US

Breast cancer incidence and death rates of patients of varying races/ethnicities.

American Cancer Society. Breast Cancer Facts and Figures 2017-2018. Atlanta: American Cancer Society, Inc.

prevalence in screening, as well as other factors that increase cancer risk, including obesity and the use of menopausal hormones. In the early 2000s, there was a decline of invasive breast cancer rates due to a publication that created awareness on the links between menopausal hormone use and diseases like breast cancer and heart disease [9].

The American Cancer Society recorded that between the years 1975 and 1989, breast cancer deaths steady climbed about 0.4% per year, but since then have dropped to 2015 for a

drastically [Figure 1.5]. This sudden rise is due to the widespread increase of mammography screening which allowed diagnosis 1 to 3 years earlier than without as well as the detection of slow growing tumors [1].

However, some of the increase is likely due to changes of reproductive patterns that are known to increase risk for breast cancer, such as having children later and having fewer

children. In the late 1980s and early 1990s, the incidence rates stabilized

briefly before rising again in the late 1990s. This further growth is seen as a result of more increases in the

total decrease in 39%. This decrease occurred in both older and younger women (though it has slowed in women younger than 50 starting in the year 2007), and reveals a total of 322,600 prevented breast cancer deaths [1]. While not entirely true for all demographic groups, a majority of this drop has been attributed to improvements in treatment and early detection [10].

Breast cancer survival rates are an estimation compared to the average survival rates of people within the same age and race groups who are not diagnosed with cancer.

The American Cancer Society's denotes the average survival rates of patients as the following: 91% within the first 5 years after diagnosis, 86% at 10 years, and 80% after 15 years [1]. Breast cancer survival is strongly determined based on the stage of the cancer after diagnosis, with the 5-year relative survival rate being 99% for when the cancer is localized in one area, 85% for a regional spread of cancer, and 27% for distant-stage cancer [11]. These rates are the average for stage of the disease and still vary greatly depending on the size of the tumor, with larger tumors

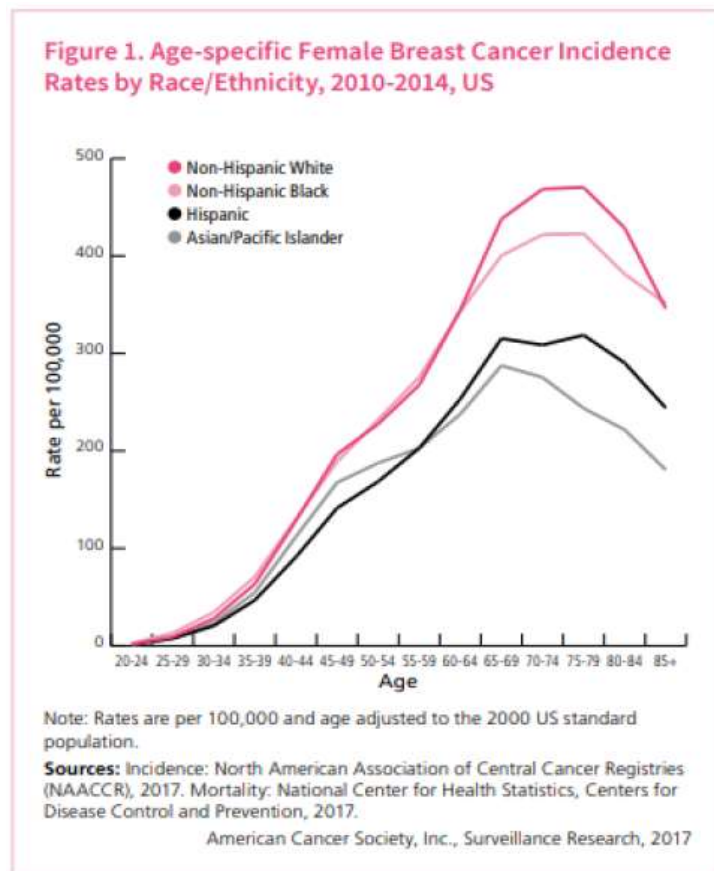


Figure 1.4: Age-specific Female Breast Cancer Incidence Rates by Race/Ethnicity, 2010-2014, US

The comparative rates of the incidence of breast cancer between patients of varying races.

American Cancer Society. Breast Cancer Facts and Figures 2017-2018. Atlanta: American Cancer Society, Inc.

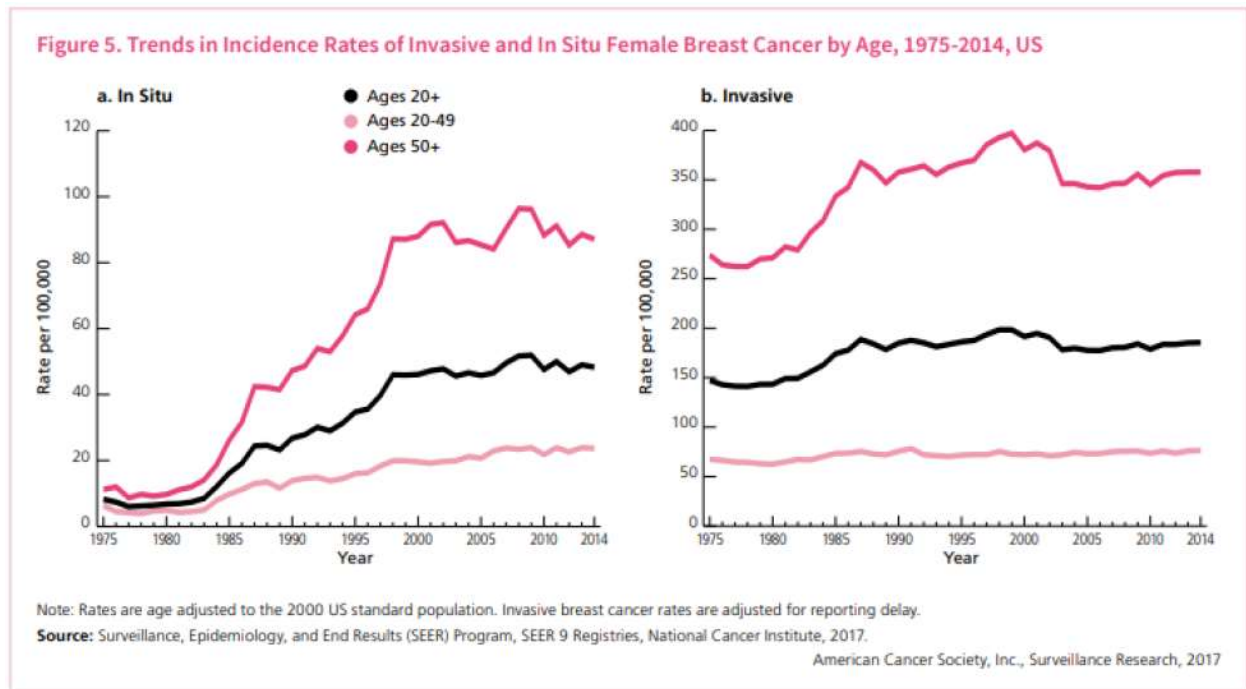


Figure 1.5: Trends in Incidence Rates of Invasive and In Situ Female Breast Cancer by Age, 1975-2014, US  
Relative rates of breast cancer incidences of differing types between women of varying age groups.  
American Cancer Society. Breast Cancer Facts and Figures 2017-2018. Atlanta: American Cancer Society, Inc.

greatly lowering survival rates. This information demonstrates the benefits of early detection of breast cancer in keeping survival rates of patients higher.

The most common methods for breast cancer screening including mammography, magnetic resonance imaging (MRI), breast ultrasound, clinical breast examination (CBE), and breast self-exams (BSE). Mammography is a low dose x-ray procedure that allows the visualization of the breast in order to diagnose and locate tumors. This can refer to the following methods of breast analysis: screen film mammography (SFM), which entails using standard x-ray equipment to record images, digital mammography (DM), which involves capturing a digital image of the breast at lower doses of radiation, and tomosynthesis, where an x-ray source travels over an arc capturing thin slices of tissue to minimize the overlapping of breast structures [12] [13]. SFM has largely been replaced by DM due to the digital method using smaller doses of

radiation as well as being significantly more accurate in women under 50 and in women with dense breast tissue [14]. Studies show that the use of breast tomosynthesis in addition to DM can be used to lower false positives as well as slightly improve cancer detection when compared to DM alone. Unfortunately, the use of tomographic images produces each 2-dimensional image separately which causes an increase in the amount of radiation dose a patient will receive from diagnosis [13]. For women that are of average risk for breast cancer, the American Cancer Society suggests that women who are over the age of 45 should be screened annually for breast cancer via mammography and should continue as long as their overall health is considered good and they have a life expectancy of over ten years [1].

Despite being the most common clinical means to observe and diagnose cancer, mammography suffers from some limitations. Mammography has been commonly shown to produce false-positive results following a women's initial screening [15]. Starting at age 40, the average patient who is screened annually for breast cancer will have a 61.3% chance of being called in for additional screening due to false positives within 10 years, and 7-9% will receive a false-positive biopsy recommendation [15]. Mammography can result in overdiagnosis of breast cancer, which refers to diagnoses of cancer that wouldn't cause a woman harm in her lifetime or that would not have progressed in the absence of screening. These overdiagnoses occur when a woman who is diagnosed dies shortly after by another illness, or when there is a non-progressive in situ or invasive cancer [1]. Another shortcoming of mammography is the dose of radiation that is administered to patients being observed, which very slightly increases risk of fatal breast cancer, about 20-25 cases in 100,000 [16]. Despite these limitations, mammography is currently considered the most effective method of detecting breast cancer in patients before any physical

symptoms manifest.

Magnetic resonance imaging (MRI), is an imaging method involving the use of powerful magnetic fields in order to noninvasively examine the human body, can be used for breast analysis. It is performed by injecting a contrast material into the body to capture images of breast tissue in high detail and help guide biopsies. MRI is recommended for use in screening women who are at an increased lifetime risk of breast cancer (greater than 25%) beginning at the age of 30; conversely, the screening is not recommended for women with a risk of cancer less than 15% [17]. Despite this, MRI is more often utilized by people who are low risk than those who are high risk [18]. The primary drawback of MRI is the high cost of screening; due to this insurance companies only cover a portion of the costs for women who are shown to be high risk [1]. Because of this expensive cost of use, MRI screening should only be used in conjunction with mammography screening.

Breast ultrasound is a means of observation that uses sound to detect lumps in breast tissue. This means of observation is used in conjunction with mammography and can detect cancer more often in patients who are known to have dense breast tissue [19]. A drawback in the use of ultrasound is that despite leading to an improved detection rate, there is a higher likelihood of receiving false-positive results which limits its utility to those with dense breasts that require additional visibility [20][21].

Clinical breast examinations (CBE) and breast self-exams (BSE) are similar in that they both involve physically feeling for lumps in breast tissue, but CBE is performed by a physician during a routine physical and BSE is to be performed by women on themselves about once a month. CBE, when performed in conjunction with a mammography, has had contradicting

results ranging from a slight increase to a significant increase in tumor detection when compared to mammography alone [22][23]. The American Cancer Society does not recommend the use of CBE for average-risk asymptomatic women due to its lack of clear benefits [1]. BSE is also not recommended by the American Cancer Society for similar reasons, however it is recommended that women become familiar with both appearance and feel of the breasts in order to determine and report if any changes have occurred [1][24][25].

Currently the primary as well as most effective means of breast cancer detection, both unaccompanied or in conjunction with another method, is mammography. While mammography is considered the most effective means of diagnosing breast cancer, there exist issues that this method of screening present. These include issues such as the patient receiving false positive diagnoses and doses of radiation that in some cases are enough to form cancer [15][16]. Mammography also presents the issue of not being fully accepted by some sociocultural backgrounds, due to a variety of reasons including fears of embarrassment, pain, cost, and potentially hearing negative results [26][27][28]. While many of these concerns are perceived, a screening option that is safer for the patient by avoiding concerns of radiation exposure would be a welcome alternative. The option of using diffuse optical spectroscopic imaging (DOSI) to receive an absorption spectrum in conjunction with spectral phasor analysis approach may offer a promising solution to these concerns that mammography presents.

## CHAPTER 2 - Novel Methods

The phasor approach is a method of mathematical analysis that involves performing Fourier transforms to sizeable stacks of data and plotting these on a scatter plot (referred to as the phasor plot) as a means of creating a simplified representation of the aforementioned data [29]. When we apply this approach to imaging analysis, it's most commonly used to evaluate observed changes over time in a stack of images; this is referred to as temporal phasor analysis and is particularly useful in fluorescence lifetime imaging microscopy (FLIM) [30]. More recently, the phasor method has been found to hold promise in spectral analysis, which we refer to as the spectral phasor approach [29]. The scope of this discussion will focus on the latter of these two approaches in terms of application in performing analyses.

The spectral phasor approach provides a simple graphical representation of complex data sets based on the absorption or emission spectrum of an image. In this method, a Fourier Transform is performed on an image or spectra in order to plot it as a single point on the phasor plot [29]. When given a stack of images or a large set of data, the phasor approach allows them

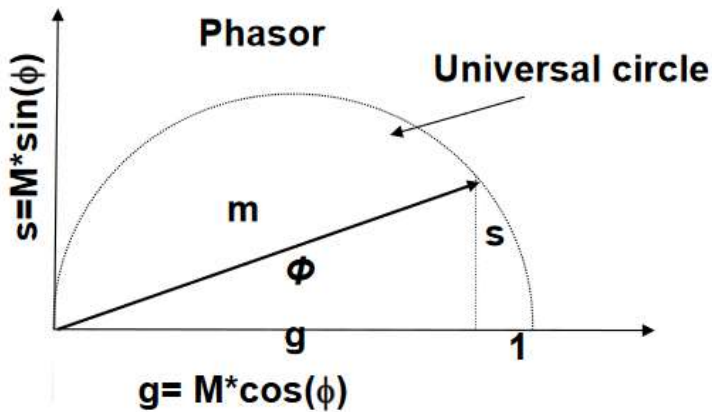


Figure 2.1: Basic Phasor Plot  
The modulus (m) and phase (phi) are shown compared to Cartesian style (s,g) coordinates. Gratton, E. The Phasor Approach to FLIM and FRET. Laboratory for Fluorescence Dynamics

to be plotted as a cluster of points on the plot. Plotting a point on the phasor plot is performed in polar coordinates, where the angle spanning from the origin is referred to as the “phase”, and the magnitude of the radius is the “modulus” [31][Figure 2.1]. The phase at which the point lies on the



plot is determined by wavelengths of the center of mass of the spectra, whereas the modulus of the phasor is based on the average width of the spectrum of each fluorescent component [31].

When the phasor approach is used to plot fluorescence lifetime or decay, a semicircle centered at position  $g = 0.5$  and  $s = 0$  with a radius of 0.5 referred to as the universal circle is utilized and the decay is expressed as a point on the chart via transform [32]. In this case, an observed lifetime would be plotted on the universal circle curve, therefore a lifetime shifted to the right result in a larger phase and decrease in modulus [Figure 2.2 a] In terms of representing the spectral phasor, the universal circle is not utilized, and instead the spectra is transformed

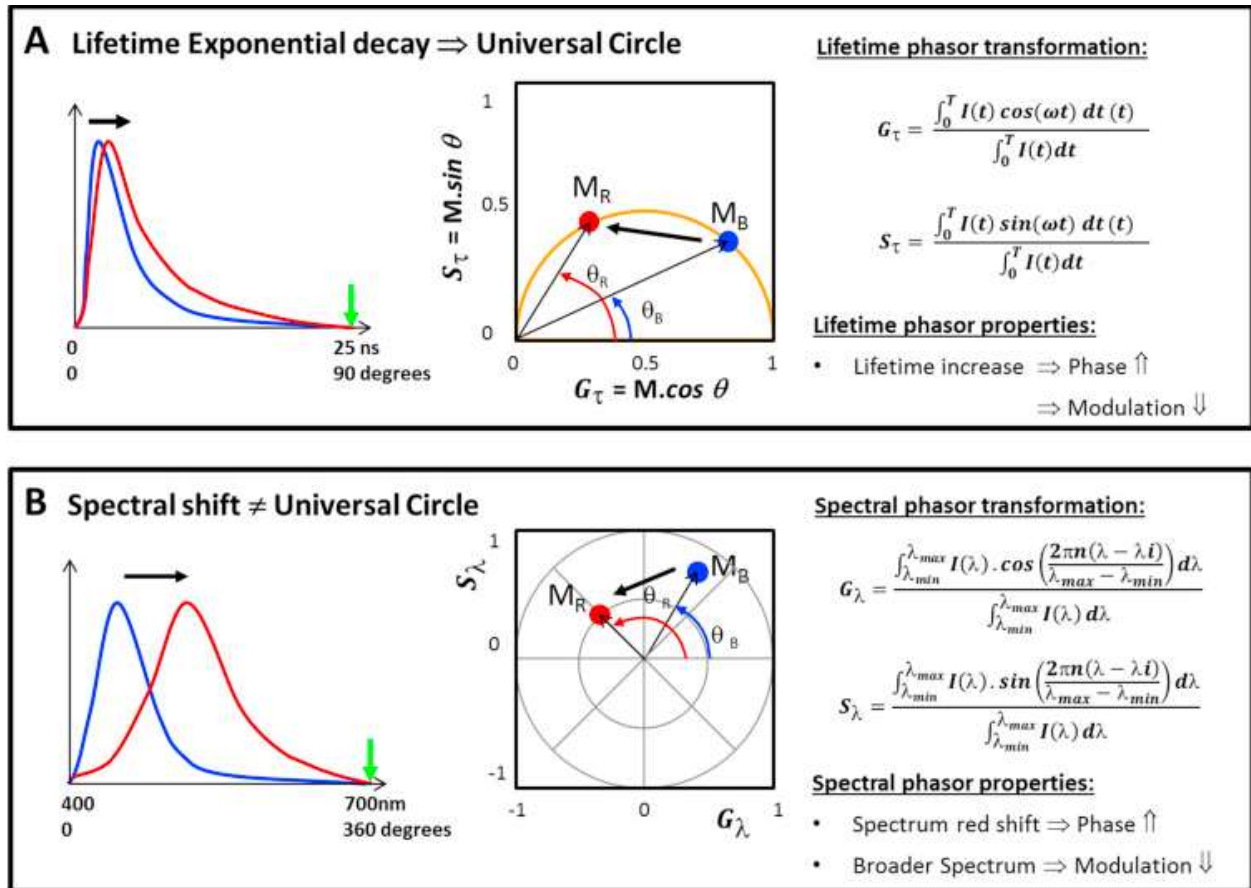


Figure 2.2: Phasor Transformations

A examples of phasor plots and the transformation equation used when observing fluorescence lifetime (a) and spectra (b).

Malacrida L, Gratton E. LAURDAN fluorescence and phasor plots reveal the effects of a H2O2 bolus in NIH-3T3 fibroblast membranes dynamics and hydration. Free Radical Biology and Medicine. 2018; 128: 144-56.

upon a circle centered at  $g = 0$  and  $s = 0$  with a radius of 1 [32]. In this case, a spectrum with a phase shifted more to the right suggests a point on the spectral phasor plot with a large phase angle from the origin as well [Figure 2.2 b]. In this way, we can see how positioning of a curve affects how it's transcribed onto the phasor plot.

The spectral phasor method of analysis benefits from being a fit-free approach which, as opposed to the use of a global fitting algorithm, the decay of each individual molecular species has its own unique representation[31][33]. This representation of raw data takes shape in the form of the spectral phasor plot. Because spectral phasor allows us to easily visualize absorption spectra as a series of clusters on the phasor plot, this approach has been utilized in visualizing biological systems such as lipids or proteins within the cell or on cell membranes [34][35]. However, there is not a significant amount of data on whether this approach can potentially be made use of in diagnostic imaging of tissues in vivo. When paired with a non-invasive imaging technique that provides us an absorption spectrum of tissue, such as diffuse optical spectroscopic imaging (DOSI), the spectral phasor approach may be able to serve as an analysis method to aid healthcare professionals with identification and detection of diseases. The goal of this is to determine spectral phasor viability in performing an analysis on and visualizing breast tissue in a variety of patients with the purpose of determining whether the approach, when paired with non-invasive and harmless means of imaging, serves as a viable option for diagnosis of breast cancer.

Our approach to determine the viability to the spectral phasor method requires us to first gather light absorption data from breast tissue of varied patients. Once that data has been obtained, the absorption values of chromophores must be analyzed in order to serve as baselines for the overall absorption of tissue. The patient data must then be analyzed via the spectral

phasor approach, which entails making use of the Fourier transform to convert the data into a single point on the spectral phasor plot. Once both of these are in place, we are able to compare the data of patients with the baseline data the chromophores have given us. Depending on where the patient data lies on the spectral phasor plot in relation to that of the chromophores, this will allow us to determine whether or not the spectral phasor approach is a feasible means of diagnosing breast cancer.

## CHAPTER 3 - Procedure

We begin our approach by receiving data from the Beckman Laser Institute regarding the absorption spectra of breast tissue from different points in numerous patients using DOSI. The patients analyzed were all women of varied ages, including groups of patients who were known to have tested positively for breast cancer and patients who were cancer free. DOSI allows us to observe individual points on a patient using a broadband lamp (~650-1000 nm) and determine the absorbance and reflectance values of these points at various points on the visible light spectrum. DOSI provides high spectral resolution in the data with a low spatial resolution of functional quantities using a handheld probe and portable interface [36]. The high spectral resolution of DOSI allows us to examine a single point on the breast and find differences in the absorption spectra by nanometer of wavelength; this allows us to observe very subtle changes in the absorption.

Before we can analyze this patient data via the spectral phasor approach, we must first observe the absorption spectra of the four most common absorbers of visible light (600 - 1000nm) in tissue: water, oxygenated hemoglobin, deoxygenated hemoglobin, and lipid [37]. Each of these chromophores absorbs one section of the visible light spectrum more readily than the other chromophores [Figure 3.1]. The purpose for determining the absorption spectra of these chromophores is to create a reference when

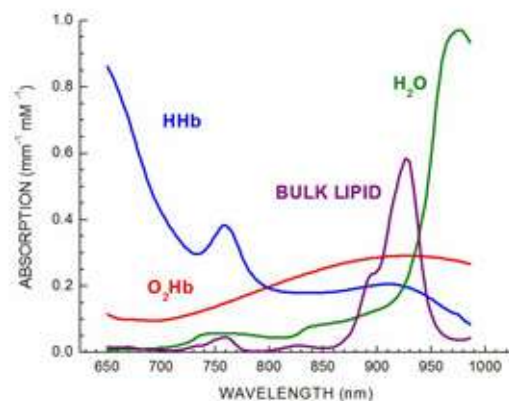


Figure 3.1: Chromophore Absorption Chart  
A graph designating the four primary chromophores in tissue, and the wavelengths of light they absorb most.  
Beckman Laser Institute and Medical Clinic,  
University of California Irvine. Research | DOSI.

analyzing data with the spectral phasor chart. These chromophores all absorb visible light at different frequencies, therefore performing spectral phasor analysis on their respective absorption coefficients throughout the entire visible light spectrum creates a single point for each chromophore on the spectral phasor chart [Figure 3.2]. When we have discovered these four points,

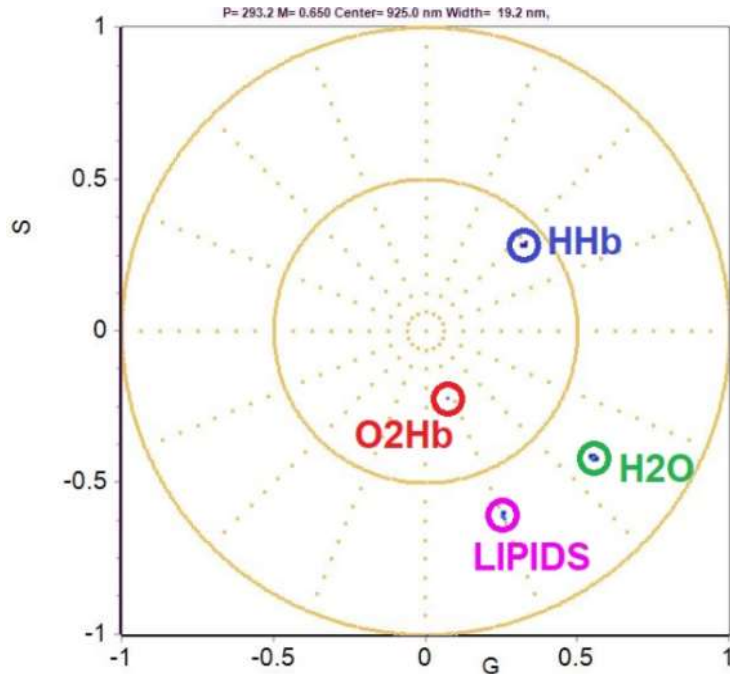


Figure 3.2: Spectral Phasor Plot with Chromophore Markers  
Basic spectral phasor plot, designating where the four primary chromophores in tissue lie.

the absorption of the light by breast tissue in female patients would also be displayed as individual points placed at varied locations on the spectral phasor plot, potentially within the region surrounded by the chromophores. When the breast tissue is examined, the particles in the tissue that will absorb light most readily will be these four chromophores. Depending on the composition of the breast tissue in patients analyzed, they should all fall within the region in areas closer to the chromophore that they have a higher composition of. For example, if a patient has dense breast tissue, which in many cases can be a signifier of a higher probability of breast cancer diagnosis, this means that the tissue in that patient is significantly more vascular than that of a patient with less dense breasts [38]. In this case, we would assume that the patients with the more vascular tissue, and therefore higher concentration of hemoglobin, both oxygenated and deoxygenated, will lie closer to where those points are in the spectral phasor plot, as opposed to a

patient with less dense breast tissue which would therefore have a higher composition of lipids.

Now that we have a baseline for various chromophores on the spectral phasor plot, we must analyze patient data and determine where the corresponding points lie on the plot. Using the absorption data for patients, we perform the Fourier transforms to convert the data into points on a phasor plot using an

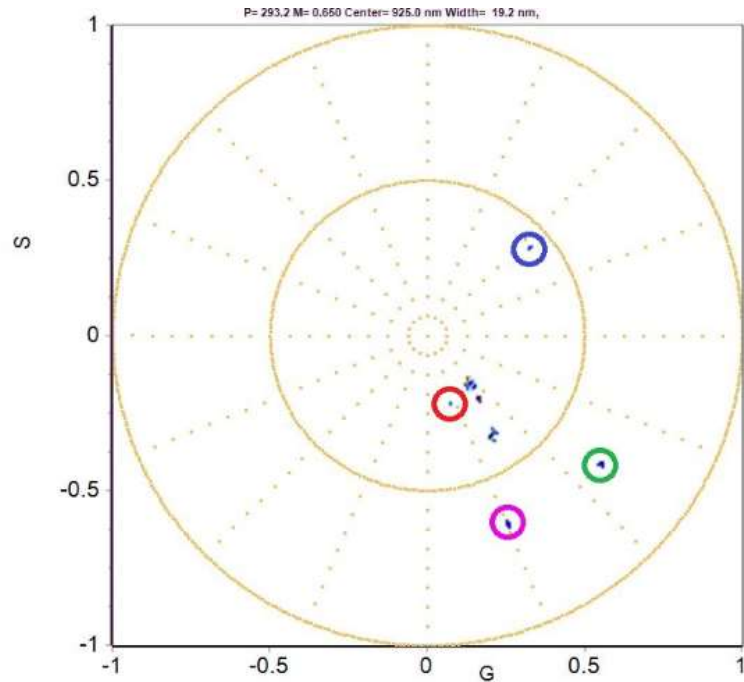


Figure 3.3: Spectral Phasor Plot with Markers and Data Points  
Spectral phasor plot with data points lying in between the region of the chromophores (designated by the encircled points). All of the experimental data points lie within the region, suggesting that they represent their proportions of chromophores within their respective tissues.

application called SimFCS. SimFCS is a software application that was designed for fluorescence image analysis, visualization, simulation, and acquisition [39]. One of many functions of this program is to help simplify the process of calculating the Fourier transform of inputted data. The program can perform these calculations on hundreds of wavelengths and their corresponding absorption spectra simultaneously, which results in a single point location on the spectral phasor plot per absorption spectra [40]. By performing this task, SimFCS allows us to seamlessly convert the absorption coefficients of the patient tissue into points on the spectral phasor plots. After performing the analysis on this patient data, we now have a spectral phasor plot containing the points that were given from the patient data along with the four points that were gathered

from the chromophore data [Figure 3.3].

Now that we have the spectral phasor plot completed with all the points from patient data, we can observe the phasor plot to determine whether this method of analysis is viable in diagnosis of breast cancer. We observe that on the plot, all the data points representing patients fall within the region where the data points of the chromophores landed. It is also worth noting that the absorption coefficients of each of the patients' breast tissue do not fall outside of the region between the four chromophores. Because breast tissue should contain some combination of the chromophores, the fact that data from all the patients have landed within these four points prove that the chromophores within the breast are the only major factors affecting absorption coefficient as well as placement on the spectral phasor plot. This analysis method may serve as a as a designator to help determine the composition of chromophores within observed tissue, which therefore may lead the way to determining a patient's likelihood of cancer.

While this can give us vague data on a patient's overall risk of breast cancer, this does not allow for determining whether certain regions in the breast tissue of a patient contains more dense vascular tissue than other points. However, because the spectral phasor approach allows us to perform analysis on large quantities of raw data, we are able to observe breast tissue in different regions on a single patient to determine local differences in absorption. Similarly to our previous approach, we analyze patients breast tissue with visible light with the goal of gathering data on the absorption coefficients at different regions to determine whether tissue contains higher concentrations of chromophores over others.

We receive data of the absorption coefficients of patient tissue data observed on multiple points in the shape of a rectangular grid-like pattern on a small area ranging between 4 and 10

centimeters in width and length. These grid patterns are observed in different areas of breast tissue of the selected patient observed. The absorption data recorded is recorded with broadband light once again, for about 700 different wavelengths between 650 and 998 nm, each of these wavelengths with a respective absorption coefficient. We then perform a spectral phasor analysis on each of the points on the grids using a program specially developed by Laboratory for Fluorescence Dynamics. Similarly to the approach used in SimFCS, the program performs

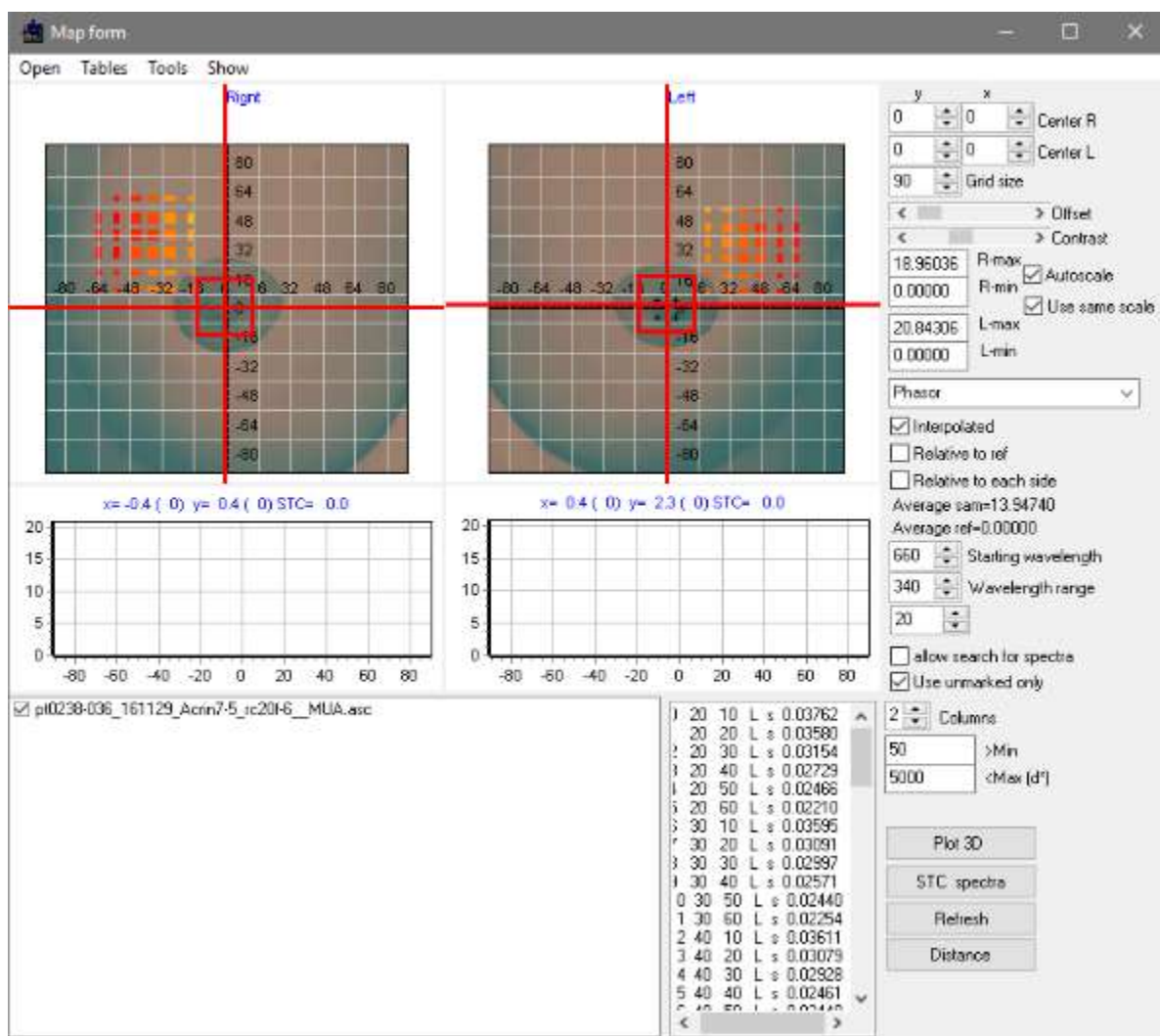


Figure 3.4: Spectral Analysis Program Results Screen

The results screen of the spectral phasor analysis screen. We can see the location of the grids analyzed in the diagram above, as well as information on the grids on the lower end of the screen.



the function to transform each of the points, but rather than plotting them all on a spectral phasor plot, it superimposes a colored grid on an image of a model of a left and right breast. This allows for visualization of the tissue composition within each section, which tells us whether or not breast tissue has a higher concentration of fatty tissue or vascular tissue [Figure 3.4].

The resulting colored grid previously noted serves as a means of analyzing chromophores observed within a localized region based on absorption coefficients of those areas. Each of the colors on the grid designates different absorption coefficients of that small region observed. Observing the types of chromophores and therefore composition of breast tissue in a small region allows us to have a clearer idea of whether or not regions are more dense and at a higher risk of breast cancer than others, which allows it to be utilized as a diagnostic tool.

## **CHAPTER 4 - Results and Discussion**

Our work on the spectral phasor approach and its ability to accurately analyze the composition of breast tissue serves as a proof of concept that this method can be used in diagnosing a patient's risk of cancer. When we analyze the data of multiple patients on a spectral phasor plot with the absorption values of the chromophores, we are able to receive values within the range of chromophores. Alongside these findings, the analysis of breast tissue from a single patient allows us a grid of varying values based on absorption of the tissue of that patient. Both of these results can be used in conjunction to determine whether or not a patient is at a significant risk of cancer.

When we analyzed the absorption coefficient of many patients with the spectral phasor approach, we are able to compare the location of patient data with that of chromophores on the phasor plot. Because the observed clusters of patient data on the phasor plots lie between the four common chromophores, oxygenated hemoglobin, deoxyhemoglobin, water, and lipids, this allows us to understand that this method determines composition of tissue based on location on the plot. The fact that none of these clusters were found outside the region between these chromophores is an indicator that the observation of absorption coefficients in this approach is not significantly swayed by factors outside of chromophore composition within the tissue. As we have previously made note, the positioning of points or clusters may suggest higher or lower concentrations of chromophores when considering their proximity to said chromophore.

The observations made via this test allows us to not only compare the positioning of the absorption coefficient to those of the chromophores, but with those of other patients as well. On the spectral phasor chart, the patient data will form clusters depending on what their observed

tissues are composed of, which we can see in [Figure 4.1]. In cases like these, we may make note of significant outliers of data that may be an indicator of unusual concentrations of chromophores for that given tissue; cases such as these potentially may be able to bring physician attention to abnormalities. As well as individual cases of unusual concentrations, this particular approach may be utilized to form a database of known dangerous

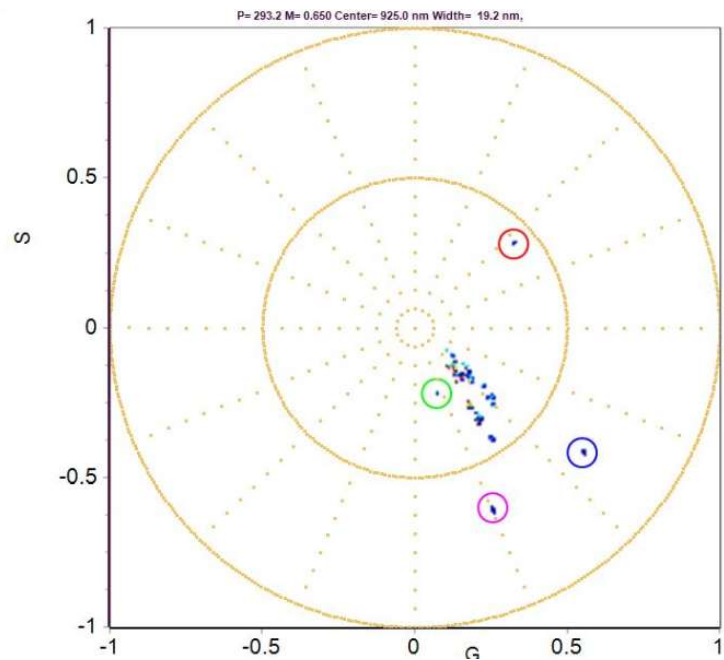


Figure 4.1: Phasor Plot with 100 Data Points

All the data points located on the phasor plot fall within a specific range, designating similarity in composition. It is unknown whether or not any patients analyzed are in healthy conditions or not.

concentrations in breast tissue. If patients who are confirmed to be positive for dense or cancerous breast tissue have their tissue observed for its absorption coefficient and plotted via the spectral phasor approach, it is possible that we may detect a region that may be an indicator of high risk of breast cancer. The utilization of a method such as this may create a highly sensitive test, where a preliminary test of absorption coefficient placement on the phasor chart could be used to determine whether a patient is high risk for cancer. In the case that a patient is high risk for cancer, this may also be an indicator that the patient will require further testing to receive a final diagnosis.

Our next attempt at observing breast tissue composition entailed use of spectral phasor analysis on a single patient to determine concentration of chromophores within a small region on

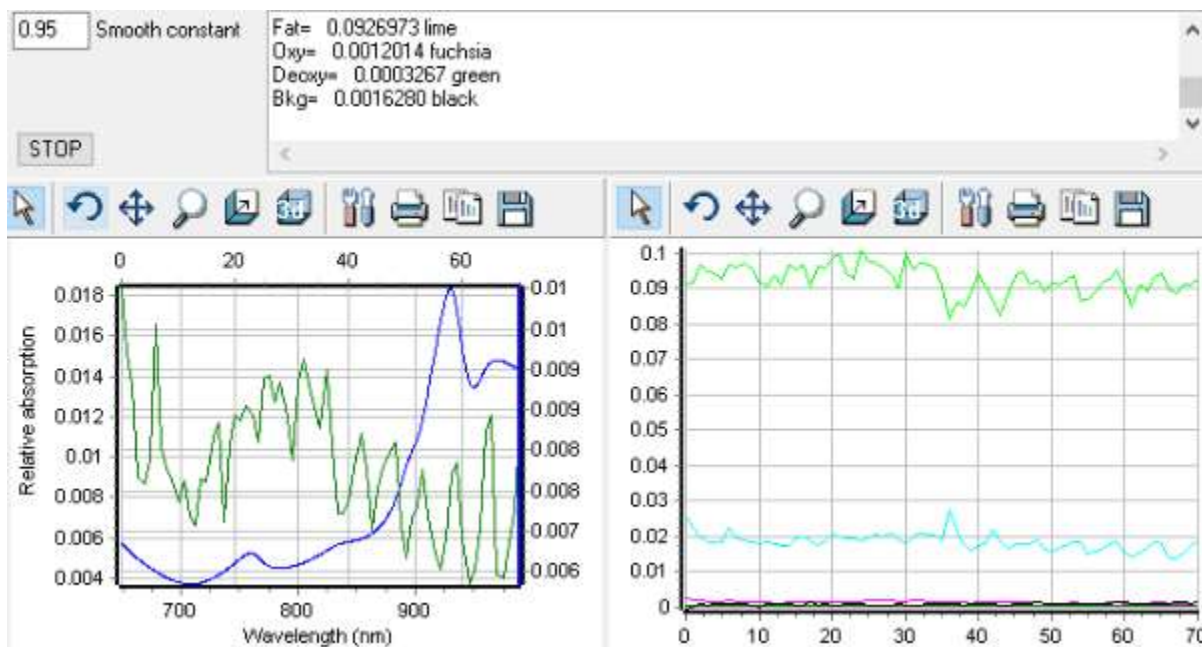


Figure 4.2: Spectral Phasor Program Analysis

A section of the main screen of the spectral phasor program. On the top, we see the proportions of the various chromophores and their colors as designated on the chart on the bottom right, which allows us to determine if breast tissue is primarily fatty or vascular. On the lower left, we have the relative total absorption over changing wavelengths.

the breast. As the program performs analysis on a given region's data and calculates the relative absorption of the region in wavelength and compares the absorption on light to specific chromophores [Figure 4.2]. This data allows us to observe whether or not the absorption of light via vascular chromophores is abnormally high, which may be an indicator of an increased risk of cancer. The resulting data also gives us a series of grids for each set of patient data, each with a heat map designating the absorption coefficient of each area in the grid and therefore tissue composition [Figure 4.3]. The figure reveals that areas that have varied compositions show significant color change on the grids, and would therefore be slightly more indicative of the possibility of a positive diagnosis. Because of the nature of this analysis of multiple smaller regions on a single patient, we are able to more thoroughly analyze tissue in order to develop a

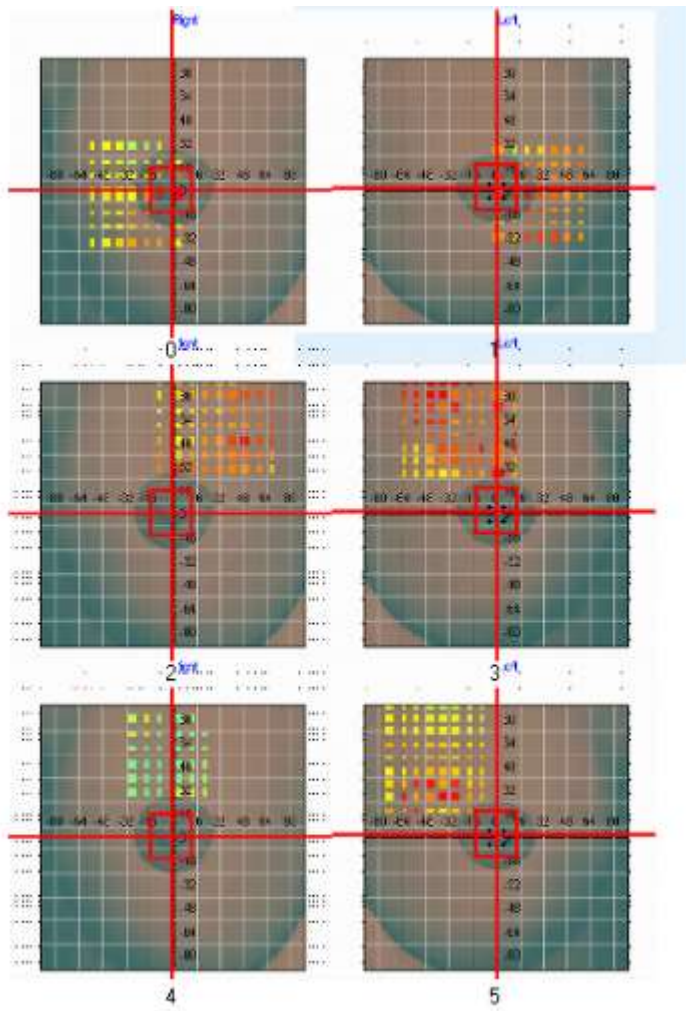


Figure 4.3: Spectral Analysis Program Resulting Grids

One of the results screens of the spectral analysis program. When multiple data sets are inputted, multiple grids are given out. Most breast tissue appears uniform when analyzed, though in situations where colors in the grid do not appear uniform, as in the bottom right, more observation may be warranted.

cancer risk. The data placement on that patient can help determine the likelihood of a patient to be at risk for cancer. Following up with patients who appear to have a higher risk of cancer would consist of observing various regions of breast tissue and receiving resulting absorption coefficients in multiple regions. When analyzed, we can find more localized areas of dense,

highly sensitive and specific

diagnostic tool.

Each of the methods we've

approached using the spectral phasor

serve a purpose for observing tissue

composition and, as a result, serving

as a diagnostic tool for patients.

However, it may be possible to use

tests in conjunction with each other in

order to more adequately perform

analysis on patients. The use of

spectral phasor for absorption values

on a single location on breast tissue

allows for a rapid and highly specific

test that can be plotted on the phasor

plot alongside data points for

chromophore absorption and known

points designating high and low

vascular tissue with the resulting grids of tissue composition. Additionally, both of these methods of analysis have the benefits of being non-invasive and harmless, as well as requiring minimal time and effort to perform given basic instructions with simple interfaces.

## **CHAPTER 5 - Conclusions**

Using spectral phasor, coupled with noninvasive imaging methods such as DOSI, as a means of diagnosing breast cancer may serve as a significant improvement when compared to current methods. Contrasting to mammography, the most commonly performed and reliable means of performing a breast cancer screening, this method of diagnosis reduces exposure to radiation, is cost-effective, and bypasses many of the perceived issues. We've determined a process by which the spectral phasor approach can serve as an examination tool when compared to numerous absorption values to observe whether or not patients fall within a range of generally healthy values or one where a patient should be met with for continued testing. In conjunction with that approach, we've detailed the ability to look at absorption values in various points across a small region in a breast to determine whether or not there are localized differences in tissue composition.

While the spectral phasor approach in conjunction with DOSI imagery has potential as a means of observation and diagnosis for breast cancer, continual refining is necessary in order to prove the approach's reliability. In order to determine values that are more closely identified to healthy patients and patients who are suffering from cancer, we require statistically significant sample sizes for each of these populations. As was briefly touched upon previously, our next steps in displaying the validity of this approach is to gather this data from numerous patients so one we have these values to create ranges on the spectral phasor plane that would be considered high risk, as well those of patients who are low risk. This requires performing DOSI imaging on numerous patients and gathering both singular and multiple readings over the grid area. When values can be predictably reproduced, the reliability of this method to make quick and accurate

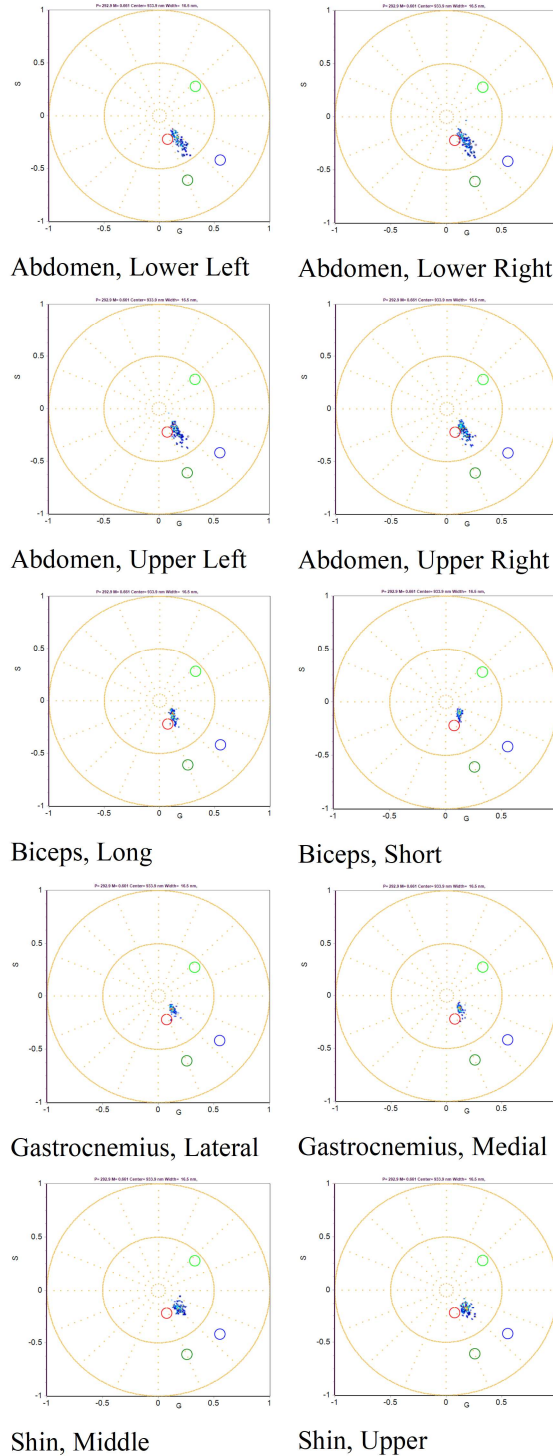


Figure 5.1: Spectral Phasor Plots of Differing Locations on Body

Spectral phasor plots with data from varying body parts observed, showing similar tissue composition. Note that differing regions on the same parts have similar clustering.

diagnoses will be proven.

In addition to its potential in diagnosing breast cancer in patients, spectral phasor may have promise in observation of other body functions and issues. Because of its functionality of analyzing copious amounts of data, which can also help with determining tissue composition, the possibility of observing tissue oxygenation in muscles exists as well. The cost-effectiveness and ability to quickly analyze multiple points of data makes spectral phasor an apt method of determining body composition in various points, as can be seen in [Figure 5.1]. In the figure, ten different body parts are analyzed in terms of their composition, and where the points lie in relation to the chromophores can reveal the body parts' relative level of tissue oxygenation (where they lie in relation to oxygenated or deoxygenated hemoglobin) or fat content



(where they lie in relation to lipids). This data reveals to us how different regions analyzed in the same body parts have clusters that lie in similar areas, while those of differing body parts suggesting similar tissue composition for different body parts. Potentially, we can also determine the degree of separation between these similar clusters using multiparametric analysis of phasor distribution. This method of analyzing phasor distribution involves determining a spectrum corresponding to each phasor plot using the maximum height of the plots' phasor distribution histograms, and then comparing these new spectrums [41]. The resulting comparison allows us to determine statistical parameters such as the area under the curve, true and false positive rates, sensitivity, and positive prediction rate [41]. Multiparametric analysis should therefore also be looked at as a possible approach for comparing breast tissue absorption based on resulting phasor plots in order to reliably determine composition and create accurate diagnoses.

There is significant potential for the spectral phasor approach in terms of analysis of non-invasive imaging or observation methods. Despite the continued effort necessary to create certainty of spectral phasor as a means of robust, accurate, and reliable breast cancer diagnostics, there exists significant promise in its ability to meet these requirements. In addition, spectral analysis paired with noninvasive and safe imaging approaches already shows the capability of addressing the concerns of the current most prominent methods of analysis. We have shown the spectral phasor approach shows promise and therefore requires continued development to meet the standards set before it.

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